6133436

SURFACE

BEAD

DNA

〜〜〜 BEAD LINKER WITH FUNCTIONAL GROUP, e.g. AMINO
••••• DNA LINKER WITH FUNCTIONAL GROUP, e.g. CARBOXY

FIG. I

FIG. 2

$R^1 = COO;(CH_2);$ (para or meta)
$R^2 = MeO; H$
$R^3 = MeO; H$
$R^4 = Cl; H$
$R^5 = (CH_2)_n; (CH_2)_n CONH(CH_2)_n$

FIG. 3

R^1=OMe;H
R^2=OMe;H
R^3=O;NH

FIG. 4

3860.20
Standard

3988.23
Iminobiotin oligo

Counts

Mass (M/Z)

FIG. 5

3687.9
Standard

3985.21
Iminobiotin oligo

Counts

Mass (M/Z)

FIG. 6

FIG. 7

PINS

BASE

PIN TOOL
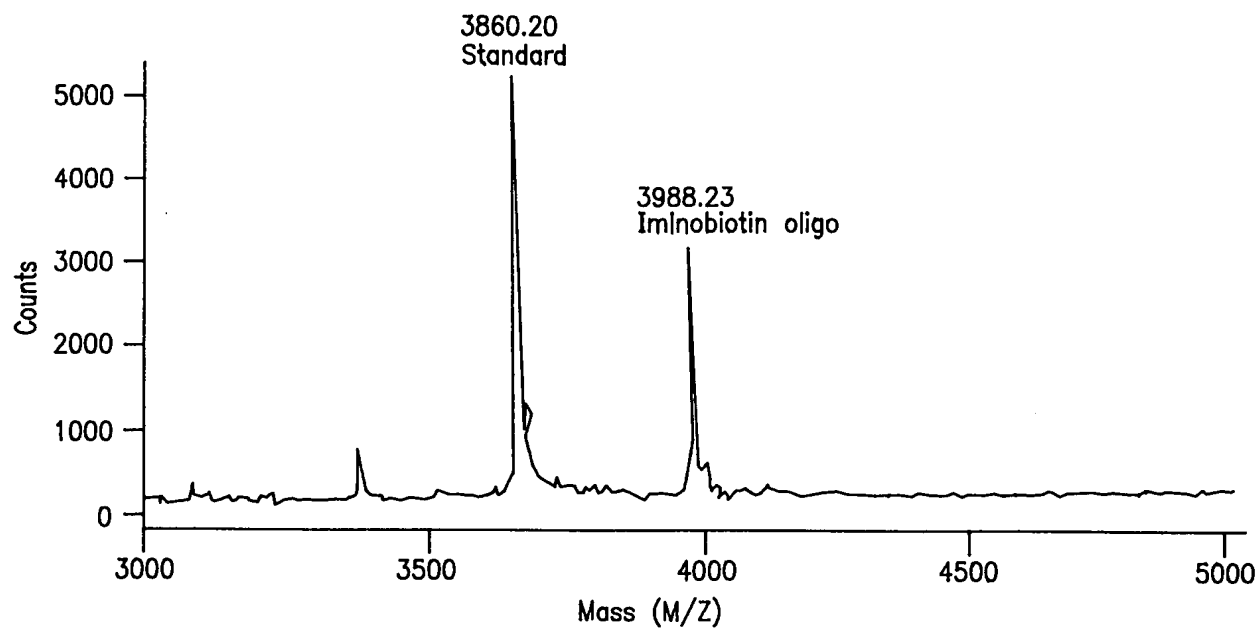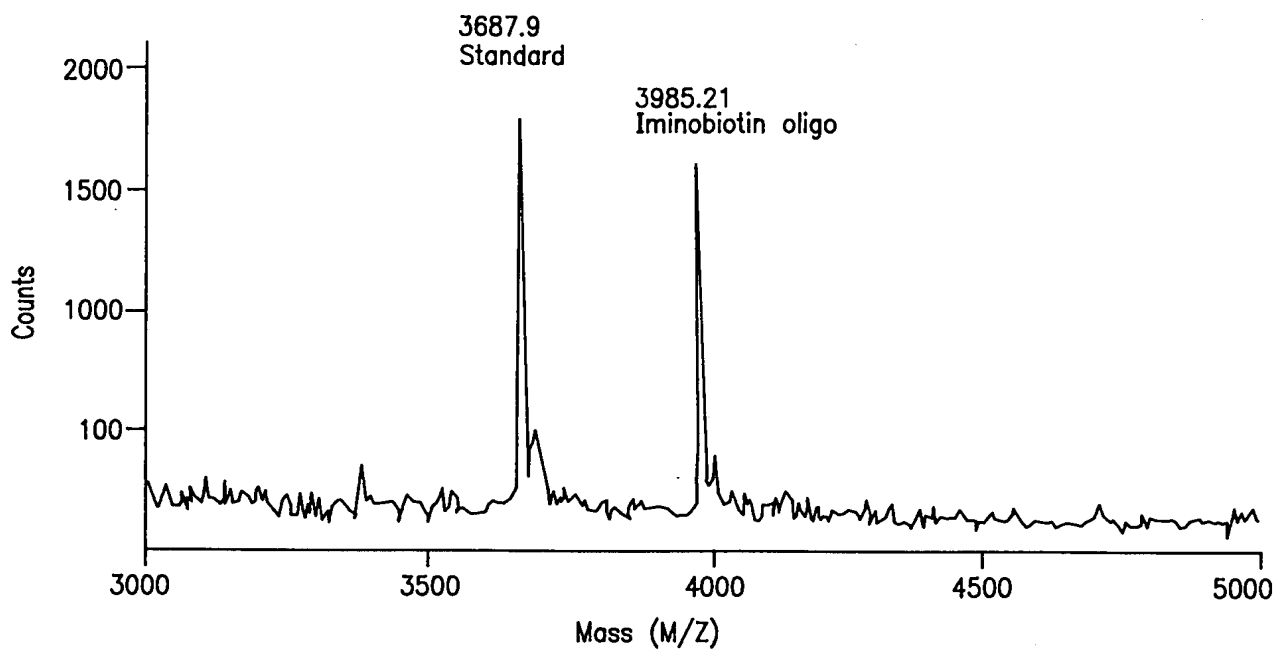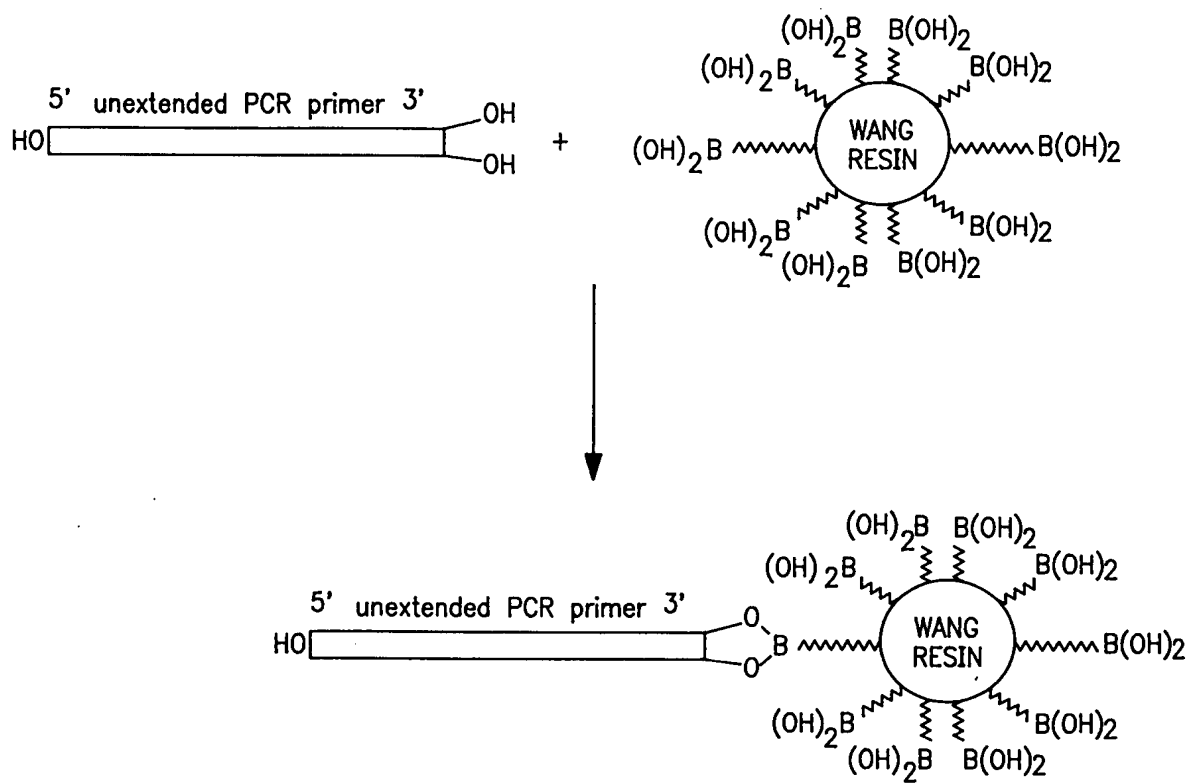
FIG. 8

FIG. 9A

FIG. 9B

FIG. 9C

FIG. 9D

FIG. 9E
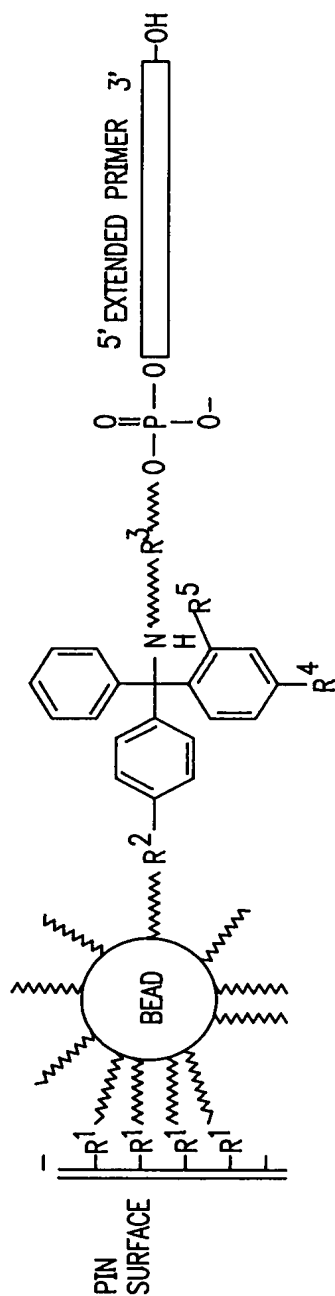
$R^1=(CH_2)_a NHCO(CH_2)_b$; $(CH_2)_c S-S(CH_2)_d$

$R^2=(CH_2)_e CONH(CH_2)_f$; $(CH_2)_g S-S(CH_2)_h$
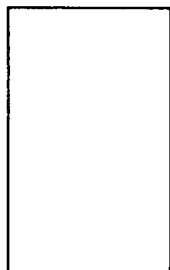
$R^3=MeO;H$

$R^4=MeO;H$

$R^5=Cl;H$

$R^6=(CH_2)_n$; $(CH_2)_x CONH(CH_2)Y$

FIG. 10

PIN
SURFACE

O
‖
O—P—O
|
O−

5'   EXTENDED PRIMER   3'

OH

• • • • magnetic interaction

——— bead functional group (streptavidin;COO⁻)

ᨸᨸᨸ DNA linker (photo—biotin: amino)
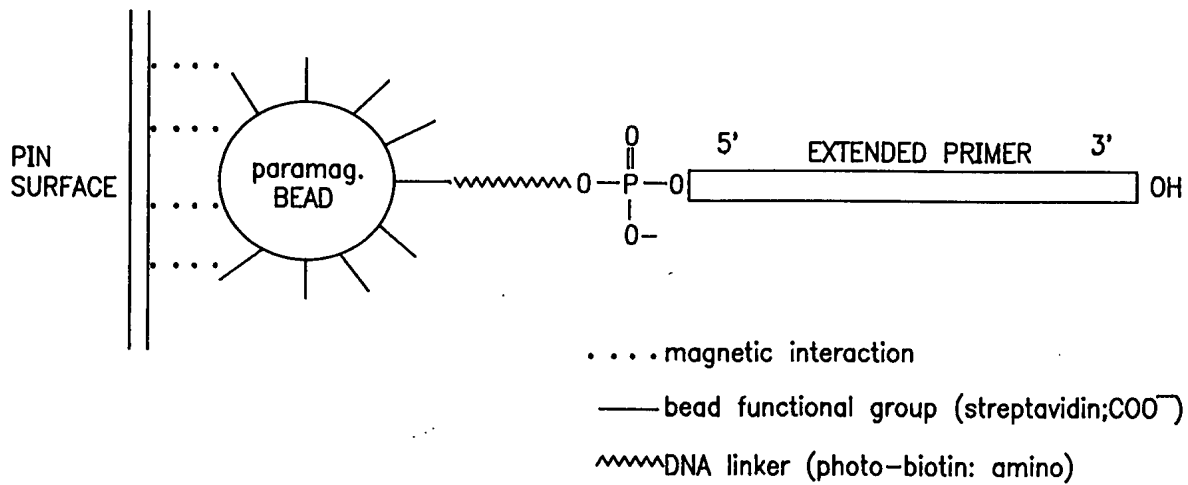
FIG. 11
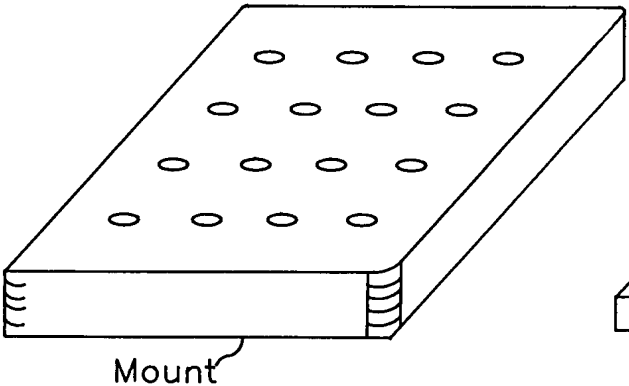
Mount

FIG. 12A

Pin tool

FIG. 12B
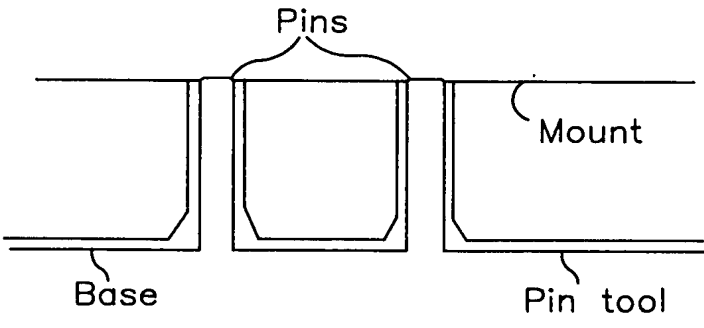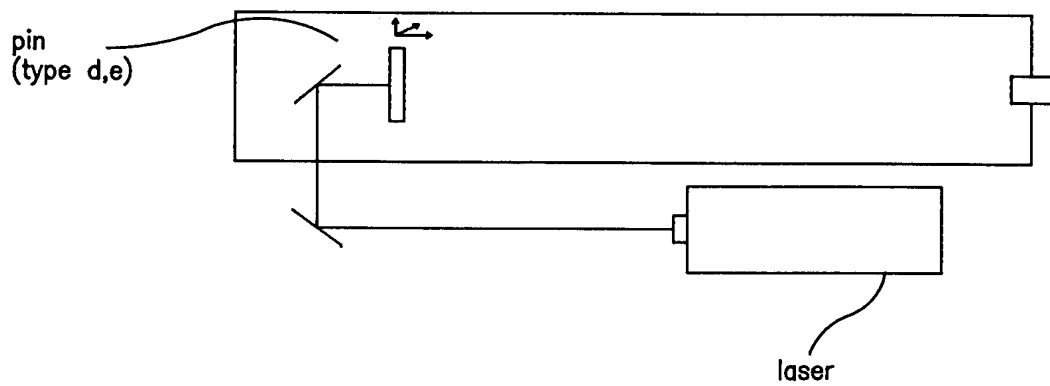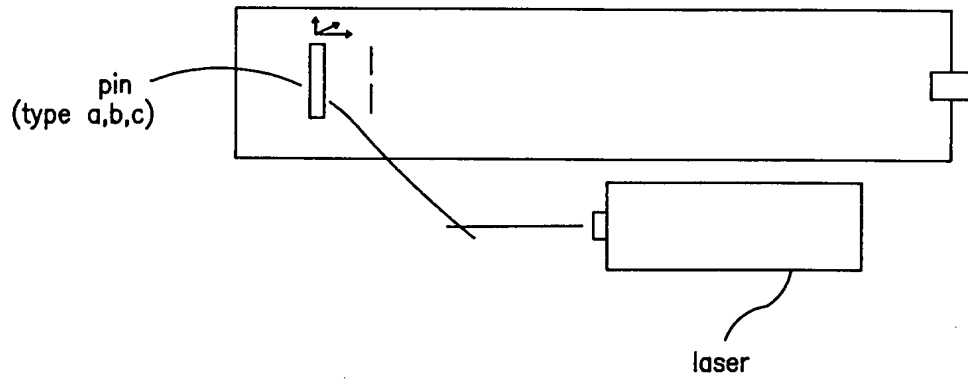
Pins

Mount

Base

Pin tool

FIG. 12C

pin
(type a,b,c)

laser

pin
(type d,e)

laser

FIG. 13

anode

cathode

solution with DNA or RNA
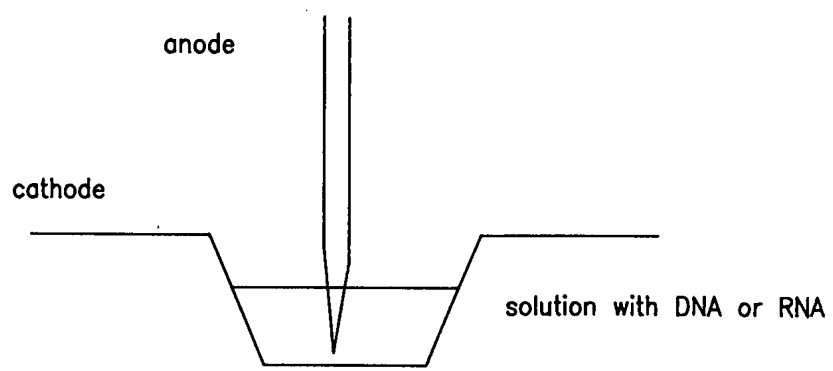
FIG. 14

Experimental flow diagram:

PCR: 50ul volume (3' modified primers)

↓

Addition of functionalized beads, incubation, capture of unused primers

↓

Aliquot PCR reaction to sequencing mixes (A,C,G,T)

↓

Aliquot sequencing mix into sequencing plate (192/384–well, 48/96 seq. R_xn's)

↓

Addition of sequencing primer(s), 5' modified to allow capture

↓

Cycle sequencing reaction: 10ul volume

↓

Application of pin tool, capture of sequencing products

↓

Wash steps (3x NH_4Cit)

↓   ↓

Addition of matrix   Removal to chips

↓   ↓

Mass spectrometry   Addition of matrix

FIG. 15